Biological Control 86 (2015) 20–27

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

Native parasitoids associated with the biological control agents of *Centaurea stoebe* in Montana, USA

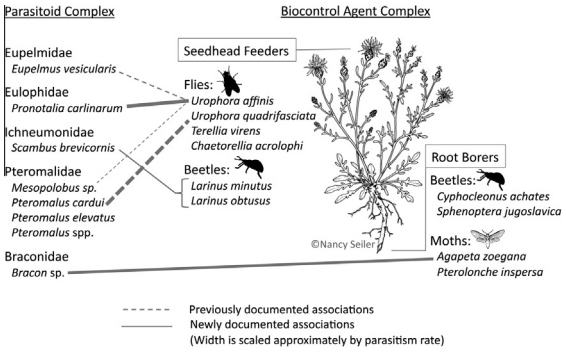
Christina R. Herron-Sweet [a,*], Jeffrey L. Littlefield [a], Erik A. Lehnhoff [a], Laura A. Burkle [b], Jane M. Mangold [a]

[a] Department of Land Resources and Environmental Sciences, Montana State University, 334 Leon Johnson Hall, Bozeman, MT 59717, USA
[b] Department of Ecology, Montana State University, 310 Lewis Hall, Bozeman, MT 59717, USA

HIGHLIGHTS

- Ten of 13 biocontrol agents introduced against *C. stoebe* were found at sites in Montana.
- Nine species of parasitoid emerged from *C. stoebe* plant material.
- Host–parasitoid associations were verified for three of the nine parasitoid species.
- Larger numbers of some parasitoid species were found compared to previous studies.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 5 November 2014
Accepted 2 April 2015
Available online 9 April 2015

Keywords:
Host–parasitoid relationship
Host-range expansion
Invasive plant
Non-target effects
Spotted knapweed
Weed control

ABSTRACT

Under classical biological control, agents are released against target non-native organisms based on the assumption that invasiveness occurs due to a lack of adapted natural enemies. Biological control agents themselves, however, can become prey to native predators, parasites and parasitoids in their introduced environment with the potential to inhibit their effectiveness. The goal of this study was to identify parasitoids that attack the biological control agents of *Centaurea stoebe* L. in western Montana, United States. Roots and seedheads of *C. stoebe* were collected from 45 sites over a two-year period, and monitored for insect emergence. Of the thirteen biocontrol agents released against *C. stoebe* in Montana, ten were reared from the plant material collected. Nine species of parasitoid emerged, four of which were previously unknown associations with these biocontrol agents: *Bracon* sp., *Pronotalia carlinarum* Szelényi & Erdös, *Eupelmus vesicularis* Retzius, *Scambus brevicornis* Gravenhorst, *Pteromalus cardui* Erdös, *Pteromalus elevatus* Walker, two unknown species of *Pteromalus*, and one unknown species of *Mesopolobus*. Host associations were determined for three of the parasitoid species, and others were inferred based on previous studies. Parasitism rates of *Urophora affinis* by *P. carlinarum* were variable by location and time of sample collection, but were surprisingly high (reaching 100% in some cases) considering this is the first record of this host–parasitoid relationship and previous studies of *U. affinis* in this region found low levels of parasitism by other species. The long-term vulnerability of biocontrol agents to parasitism and predation by native organisms is a concern for the practice of classical biological control, especially for agents that have been established for several decades, and thus merits further research attention.

* Corresponding author. Fax: +1 (406) 994 3933.
E-mail addresses: c.herronsweet@gmail.com (C.R. Herron-Sweet), jeffreyl@montana.edu (J.L. Littlefield), erik.lehnhoff@montana.edu (E.A. Lehnhoff), laura.burkle@montana.edu (L.A. Burkle), jane.mangold@montana.edu (J.M. Mangold).

http://dx.doi.org/10.1016/j.biocontrol.2015.04.001
1049-9644/

1. Introduction

Insects selected as biological control agents are often hosts to a complex of parasitoids in their native range (Mills, 2009). During importation and release of a new biological control agent, measures are taken to ensure that agents are free of parasitoids, pathogens, and other contaminating organisms (Turner et al., 1990). This is important because agents in their introduced range are more likely to be effective in areas where their own natural enemies are absent (Boughton et al., 2012). Unfortunately, biological control agents may not always remain enemy-free because introduced organisms can become prey to native predators or parasitoids in their new environment (Cornell and Hawkins, 1993).

Parasitism and predation by native organisms was listed as one of the top three causes of biological control agent failure in a review of 119 failed insect pest control programs (Stiling, 1993). Parasitism of weed biological control agents can also be common. Hill and Hulley (1995) found that in South Africa 40% of established weed biocontrol agents were hosts to native parasitoids, and in New Zealand Paynter et al. (2010) found 10 out of 28 agents were parasitized. Although the South Africa study found no strong effect of parasitoids on the success of biocontrol, the New Zealand survey found a significant association between parasitism and failure of agents to suppress their target weed. Although there has not been a similar comprehensive study of weed biological control programs in the United States, parasitism of weed biocontrol agents has been reported in numerous cases (Boughton et al., 2012; Lang and Richard, 1998; Lang et al., 2003; Littlefield, 1991; Swope and Satterthwaite, 2012; Wehling and Piper, 1988).

Biological control of the rangeland invasive plant *Centaurea stoebe* L. (Fam.: Asteraceae) began in the 1970s. *C. stoebe* is one of the most widespread and problematic invasive plants in the western United States and Canada, currently reported in seven Canadian provinces and all but three of the lower 48 states (United States Department of Agriculture, 2013). It is listed as a noxious weed in 14 states and four of the six southernmost Canadian provinces (Rice, 2014). Montana had the largest infested area in the northwestern states in the early 1990s (Müller-Schärer and Schroeder, 1993). Since 1973, thirteen biological control agents have been released for the control of *C. stoebe*, including eight flower head feeders and four root herbivores (Winston et al., 2010).

Field studies on the biological control agents of *C. stoebe* in their native range have reported parasitism rates ranging from 10% to 60% (Groppe, 1992, 1990; Müller et al., 1988; Stinson, 1987). Relatively little is known about parasitoids attacking *C. stoebe* biological control agents in their introduced range and the degree to which they may limit biocontrol efficacy. Seven studies investigating parasitism of *C. stoebe* biocontrol agents in the United States have been published and all were focused on *Urophora affinis* Frauenfeld and *Urophora quadrifasciata* Meigen (Diptera: Tephritidae), the earliest agents released (Table 1). Only two studies included sites in Montana (Lang and Richard, 1998; Turner et al., 1990) and these both were conducted over 15 years ago. Although parasitoid abundance and parasitism rates have been geographically variable (Table 1), it is plausible that parasitism of *C. stoebe* biocontrol agents has the potential to decrease populations and impede control efficacy since parasitism rates of *U. affinis* in some past studies were quite high. Given the limited scope of previous work and the potential impact to biocontrol efficacy, further investigation into parasitism of the *Urophora* spp. and other *C. stoebe* biocontrol agents is warranted. The goals of this study were: (1) to identify larval/pupal parasitoids attacking introduced biological control agents of *C. stoebe* in Montana and (2) to determine the percent parasitism of any host–parasitoid associations found. Since exotic organisms can accumulate natural enemies over time in their new range (Cornell and Hawkins, 1993; Flory and Clay, 2013), and no work has been conducted to determine the extent of parasitism for root feeders or more recently introduced agents of *C. stoebe*, we expected to find a greater diversity of parasitoids on the *Urophora* spp. compared to earlier studies, and uncover new associations for other agents.

Table 1
Parasitoids associated with *Centaurea stoebe* biological control agents in their introduced range described in the literature to date. Each study used a different metric to quantify parasitoid(s) found. The relative abundance or impact of these reported metrics have been placed under the column "parasitoid abundance or parasitism rate". In this column, "combined parasitism" refers to the combined parasitism rate of multiple parasitoids on a single host from the same study. If multiple states are listed as field site locations, calculations were made across states and an asterisk(*) indicates presence in that state.

Parasitoid	Biocontrol agent host	Location	Parasitoid abundance or parasitism rate	Source
Eupelmus sp. possibly *melanderi* or *vesicularis* (Eupelmidae)	*Urophora affinis*	Idaho	<3% combined parasitism	Gillespie (1983)
Pteromalus sp. (Pteromalidae)	*U. affinis*	Idaho	<3% combined parasitism	Gillespie (1983)
Tetrastichus sp. (Eulophidae)	*U. affinis*	Idaho	<3% combined parasitism	Gillespie (1983)
Microdontomerus anthonomi Crawford (Torymidae)	*U. affinis*	Montana*, California	1.3% parasitism	Turner et al. (1990)
Pteromalus sp. (Pteromalidae)	*U. affinis*	Montana*, Washington*, Wyoming, Minnesota, Nebraska, Wisconsin, South Dakota, Arizona	Present at 4 of 65 collection sites	Lang and Richard (1998)
Microdontomerus anthonomi Crawford (Torymidae)	*U. affinis*	Montana*, Washington*, Wyoming, Minnesota, Nebraska, Wisconsin, South Dakota, Arizona	Present at 2 of 65 collection sites	Lang and Richard (1998)
Mesopolobus sp. (Pteromalidae)	*U. affinis*	Montana*, Washington, Wyoming, Minnesota, Nebraska, Wisconsin, South Dakota, Arizona	Present at 1 of 65 collection sites	Lang and Richard (1998)
Pteromalus sp. (Pteromalidae)	*U. quadrifasciata*	Michigan	60% parasitism	Lang et al. (2003)
Pteromalus cardui Erdos (Pteromalidae)	*U. quadrifasciata*	Tennessee	33.5% combined parasitism	Kovach (2004)
Brasema sp. (Eupelmidae)	*U. quadrifasciata*	Tennessee	33.5% combined parasitism	Kovach (2004)
Eurytoma sp. (Eurytomidae)	*U. quadrifasciata*	Tennessee	33.5% combined parasitism	Kovach (2004)
Pteromalus cardui Erdos (Pteromalidae)	*U. quadrifasciata*	Michigan	Found at 9 out of 10 sites	Marshall et al. (2004)
Aprostocetus sp. (Eulophidae)	*U. affinis*	Michigan	No effect on fly presence or populations	Marshall (2007)

2. Materials and methods

2.1. Seedhead collection and insect emergence

To sample flower-feeding biocontrol agents and any associated parasitoids, we collected seedheads in August 2012 (33 sites), late September through mid-October 2012 (28 sites), and April 2013 (26 sites). Different sampling dates were intended to target larval/pupal life stages of different biocontrol agents (i.e., August to capture the *Larinus minutus* Gyllenhal and *L. obtusus* Hochhuth (Coleoptera: Curculionidae) and first generation of flies, and September–October to capture the second generation of flies) and to evaluate parasitism and emergence during different seasons. In total, we collected seedheads from 45 sites, some on all three collection occasions but others only once or twice (Fig. 1A, Table S1 in the online supplement).

Approximately 200 *C. stoebe* seedheads (one to three per plant) were clipped from plants at each site and placed in 0.5 L clear plastic cups with mesh tops and held in the laboratory. We monitored insect emergence from seedheads collected in 2012 throughout the autumn, capturing all adult insects with an aspirator and preserving them in 70% ethanol. In November, we placed seedheads in a refrigerator at ~4 °C for overwintering. In March 2013, they were returned to room temperature and monitored again for emergence until June, along with the seedheads collected in April 2013.

In August and September 2013, we re-sampled nine of the initial sites where parasitoids had been most abundant in the previous year. Rearing methods were altered in order to help associate parasitoid species with specific biocontrol agents. Fifty of the 200 seedheads from each site were placed into individual 30 ml plastic snap-top containers. Where parasitoids emerged, seedheads were dissected to find evidence of a host. The remaining seedheads were frozen for later dissection (see Section 2.3).

2.2. Root collection and insect emergence

We collected roots of *C. stoebe* between 20 June and 16 July in 2012 and 2013 prior to the seasonal emergence of root-feeding biocontrol agents. In 2012, collections were taken from 19 sites in western Montana (Fig. 1B, Table S2 in online supplement), and in 2013, roots were only collected from the two sites where parasitoids were found in 2012. At each site, approximately 50 large *C. stoebe* plants were dug or pulled from the ground. We specifically selected large plants because root biocontrol agents tend to attack larger plants (Story and Stougaard, 2006). Aboveground herbage was removed and roots were placed into black plastic trash bags for transport to the laboratory. Root material was kept moist until it could be placed in rearing containers.

We placed roots collected in both years into 20 L mesh bags with a mixture of vermiculite and soil (equal mix of sand, loam, and peat moss) or in 80 cm × 25 cm × 5 cm plastic trays, covered with a thin layer of vermiculite plus soil, and then sealed with a clear lid and kept moist. All roots in a single bag or tray originated from the same site, but often multiple bags or trays were required to fit all roots collected from a single site. Rearing containers were housed in a greenhouse set at ~24 °C with ambient photoperiod and inspected once every two to three days for adult emergence. Insects were killed by freezing, and preserved in 70% ethanol.

In November 2012, summer collected roots were transferred to resealable plastic bags with some soil, moistened, and stored in an indoor unheated storage space to simulate winter temperatures. In March 2013, we removed these roots from storage and monitored them for additional emergence. Roots collected in June 2013 were monitored through the summer and into autumn, after which roots from trays or bags where parasitoids had emerged were dissected to determine parasitoid–host associations (see Section 2.3).

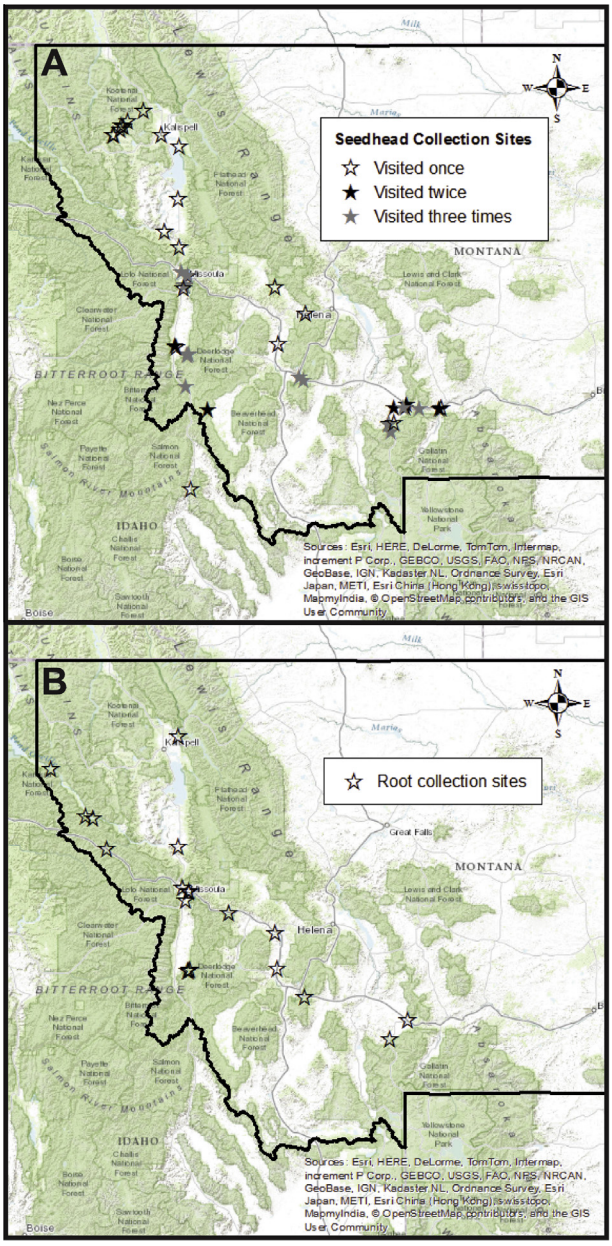


Fig. 1. Sites of *Centaurea stoebe* (A) seedhead and (B) root collections made in western Montana during 2012 and 2013. Seedhead sites are coded by how many times they were visited to make collections. GPS coordinates and collection dates for the sites can be found in Tables S1 and S2 in the online supplement.

2.3. Plant material dissection

Dissections of plant material were undertaken to determine parasitoid – biological control agent associations. We dissected all roots and subsamples of 15–25 seedheads from mass rearing containers where parasitoids had emerged, and all seedheads in individual containers from which parasitoids emerged. During dissection, all evidence of biological control agents or parasitoids was recorded. We often froze seedheads before dissection in order to kill mites (Acari: Pyemotidae) associated with *Urophora* spp.

2.4. Percent parasitism calculation

When a host–parasitoid relationship was determined, we calculated an approximate percent parasitism at each site based

on biocontrol agent and parasitoid emergence from the mass rearing containers (200 seedheads, or ~50 roots). Mean percent parasitism within each of the sampling dates across all sites was then calculated, excluding sites with no parasitism. While an exact percent parasitism rate would be found by dividing the number of parasitized host individuals by the original total number of host individuals present, we estimated the number of parasitized host individuals as the number of parasitoid individuals that emerged if it was a solitary parasitoid. Alternatively, if it was a gregarious parasitoid, the total number of emerged parasitoid individuals divided by the mean number of parasitoid individuals that attacked a single host individual (obtained from plant material dissections and averaged over all sampling dates). The original number of host individuals was estimated by adding the number of host individuals that emerged and the estimated number of parasitized host individuals. Thus, estimates of percent parasitism are approximate since they are based on relative abundances of parasitoids and biocontrol agents, rather than explicit measurements of parasitism and mortality (van Driesche, 1983).

3. Results

3.1. Biological control agents

Five seed-feeding biological control agents were reared from seedhead collections: *U. affinis*, *U. quadrifasciata*, *Chaetorellia acrolophi* White & Marquardt, *Terellia virens* Loew (Diptera: Tephritidae) and *Larinus* spp. (Coleoptera: Curculionidae) (Table 2). We did not differentiate *L. minutus* from *L. obtusus*, but it is likely that we had a mix of both species since both are established in Montana. Of the seed-feeding agents, *U. affinis* was the most numerous and *T. virens* the least, being found at only seven sites in very low numbers. Four root-feeding biological control agents emerged from the root collections: *Cyphocleonus achates* Fahraus (Coleoptera: Curculionidae), *Agapeta zoegana* L. (Lepidoptera: Tortricidae), *Sphenoptera jugoslavica* Obenberger (Coleoptera: Buprestidae) and *Pterolonche inspersa* Staudinger (Lepidoptera: Pterolonchidae) (Table 2). The last two were present in very low numbers, with three and one individuals, respectively, and were each found at different sites.

Table 2
Summary of the insect emergence from *Centaurea stoebe* seedhead and root collections including the species found, the percentage of sites where they occurred, the mean abundance of individuals (±SD, unless it was only present at one site), parasitoid–host relationships, and percent parasitism when applicable.

Species	% of sites present	Mean number adults emerged per site (when present)	Associated parasitoid[a]	Mean% parasitism[b]
Seedhead collections August 2012 (33 sites, 200 seedheads per site)				
Biocontrol agents				
Chaetorellia acrolophi	87.9	20.5 ± 19.5		
Larinus spp.	81.8	10.8 ± 9.9	*Scambus brevicornis*	
Terellia virens	18.2	8.5 ± 15.9		
Urophora affinis	100.0	46.3 ± 39.7	*Pronotalia carlinarum*	20.5 ± 24.8
Urophora quadrifasciata	39.4	7.8 ± 11.6		
Parasitoids				
Pronotalia carlinarum	84.8	193.3 ± 364.6		
Pteromalinae spp.[c]	63.6	14.6 ± 29.9		
Seedhead collections Sept–Oct 2012 (28 sites, 200 seedheads per site)				
Biocontrol agents				
Chaetorellia acrolophi	25.0	2.6 ± 1.9		
Larinus spp.	17.9	1.6 ± 0.9	*Scambus brevicornis*	
Terellia virens	14.3	1.3 ± 0.5		
Urophora affinis	92.9	29.0 ± 29.5	*Pronotalia carlinarum*	26.5 ± 34.4
Urophora quadrifasciata	39.3	2.4 ± 1.8		
Parasitoids				
Pronotalia carlinarum	85.7	63.5 ± 102.5		
Pteromalinae spp.	50.0	7.7 ± 11.8		
Scambus brevicornis	7.1	1.0 ± 0.0		
Seedhead collections May 2013 (26 sites, 200 seedheads per site)				
Biocontrol agents				
Chaetorellia acrolophi	46.2	2.7 ± 2.9		
Terellia virens	3.8	3.0		
Urophora affinis	73.1	74.0 ± 41.9	*Pronotalia carlinarum*	34.2 ± 41.6
Urophora quadrifasciata	11.5	3.0 ± 3.5		
Parasitoids				
Pronotalia carlinarum	84.6	107.7 ± 116.1		
Pteromalinae spp.	92.3	6.3 ± 6.5		
Scambus brevicornis	15.4	2.5 ± 1.9		
Eupelmus vesicularis	19.2	1.6 ± 0.5		
Root collections June–July 2012 (19 sites, ~50 roots per site)				
Biocontrol agents				
Agapeta zoegana	89.5	6.9 ± 7.5	*Bracon* sp.	20% and 29%[d]
Cyphocleonus achates	57.9	2.9 ± 3.3		
Pterolonche inspersa	5.3	1.0		
Sphenoptera jugoslavica	5.3	2.0		
Parasitoids				
Bracon sp.	10.5	1.5 ± 0.7		

[a] Blank spaces indicate undetermined host–parasitoid associations.
[b] Mean parasitism calculations exclude sites with no parasitism. Blank spaces indicate not enough information to calculate a percent parasitism.
[c] Includes *Mesopolobus* sp. and *Pteromalus* spp.
[d] Only two sites had parasitoids, percentages are for each site.

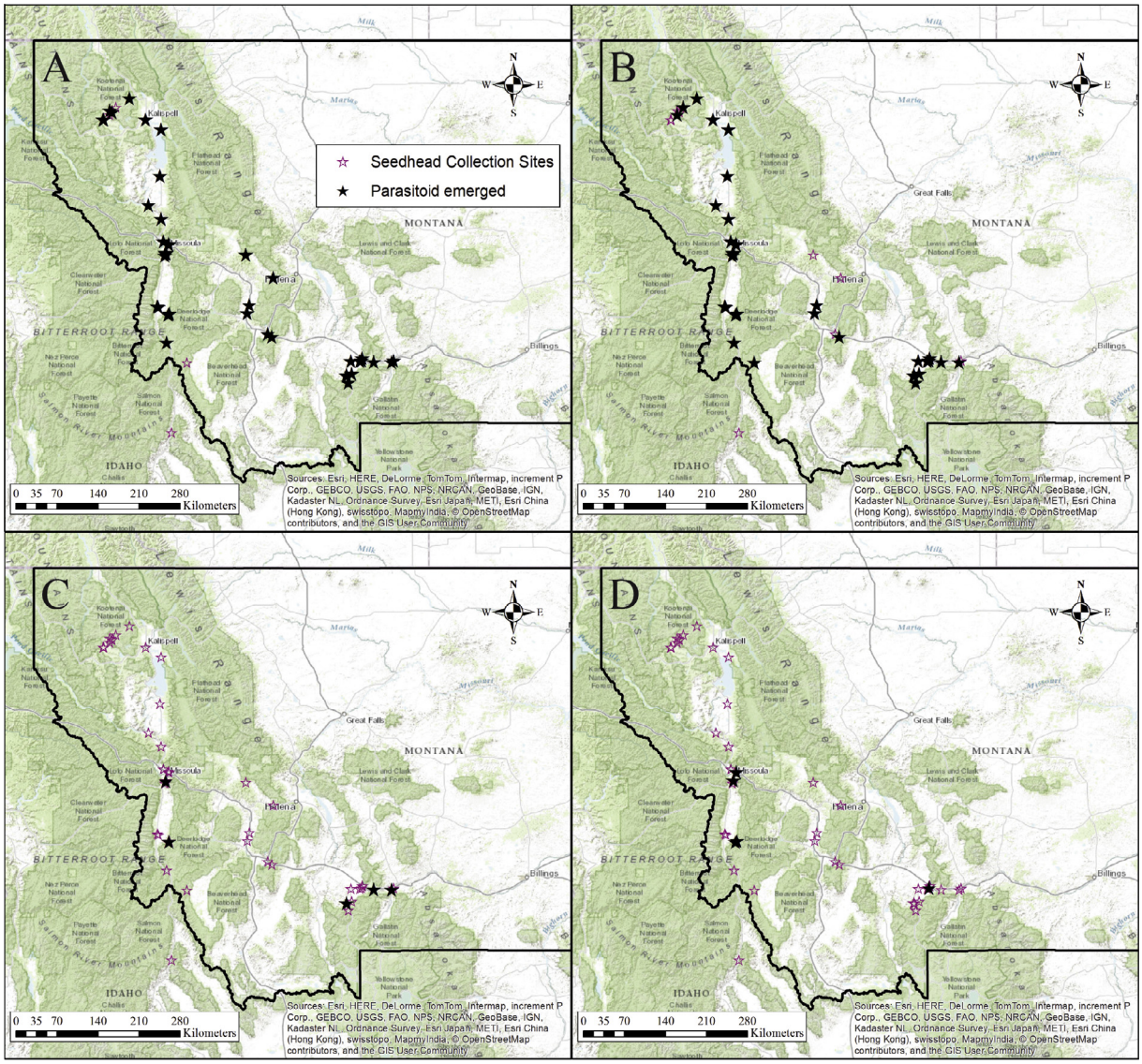


Fig. 2. *Centaurea stoebe* seedhead collection sites highlighting where parasitoids were present: (A) *Pronotalia carlinarum*, (B) Pteromalinae spp., (C) *Eupelmus vesicularis*, and (D) *Scambus brevicornis*. Solid stars indicated where parasitoids were present.

3.2. Parasitoids

Nine species of hymenopteran parasitoid emerged from the plant material. *Bracon* sp. (Hymenoptera: Braconidae) was the only parasitoid to emerge from roots (Table 2). *Pronotalia carlinarum* Szelényi & Erdös (Hymenoptera: Eulophidae), *Eupelmus vesicularis* Retzius (Hymenoptera: Eupelmidae), *S. brevicornis* Gravenhorst (Hymenoptera: Ichneumonidae), and five species of Pteromalinae (Hymenoptera: Pteromalidae) emerged from seedheads (Table 2). Almost all of the Pteromalinae individuals reared were from the genus *Pteromalus*, but one unknown species of *Mesopolobus* was encountered, albeit rarely (referred to collectively from here on as Pteromalinae spp.). The *Pteromalus* species included *Pteromalus cardui* Erdös, *Pteromalus elevatus* Walker, and two unknown species. In addition, what we referred to as *S. brevicornis* may represent two species; one male specimen appeared different from others, but could not be differentiated using diagnostic keys for males (Wahl, personal communication).

P. carlinarum and Pteromalinae spp. emerged from nearly all 45 sites, and from all three collection dates (Fig. 2A and B, respectively, Table 2). *P. carlinarum* had the highest overall emergence, with one collection site in Lake County in northwestern Montana yielding over 1400 individuals from 200 seedheads. In contrast, *E. vesicularis* only emerged from the spring collections, with only one or two individuals at each of five sites (Table 2 and Fig. 2C). *S. brevicornis* was reared only from the late autumn and spring collections, with less than five individuals at each of five sites (Table 2 and Fig. 2D).

3.3. Host–parasitoid associations and percent parasitism

Seedhead dissections revealed no host associations with either *E. vesicularis* or any of the Pteromalinae species, however we found that *P. carlinarum* parasitized *U. affinis*. Host affiliation for *Bracon* sp. and *S. brevicornis* was slightly less certain, but based on the evidence from dissections, it appeared that *Bracon* sp. was associated with *A. zoegana*, and *S. brevicornis* with *Larinus* spp. The following sections describe each association in more detail (also see Herron-Sweet, 2014).

3.3.1. P. carlinarum

Seedhead dissections indicated that *P. carlinarum* is a gregarious parasitoid that attacks the larvae of *U. affinis*. Thirteen of 145 seedheads had one or two *U. affinis* galls that contained 1–15 larval,

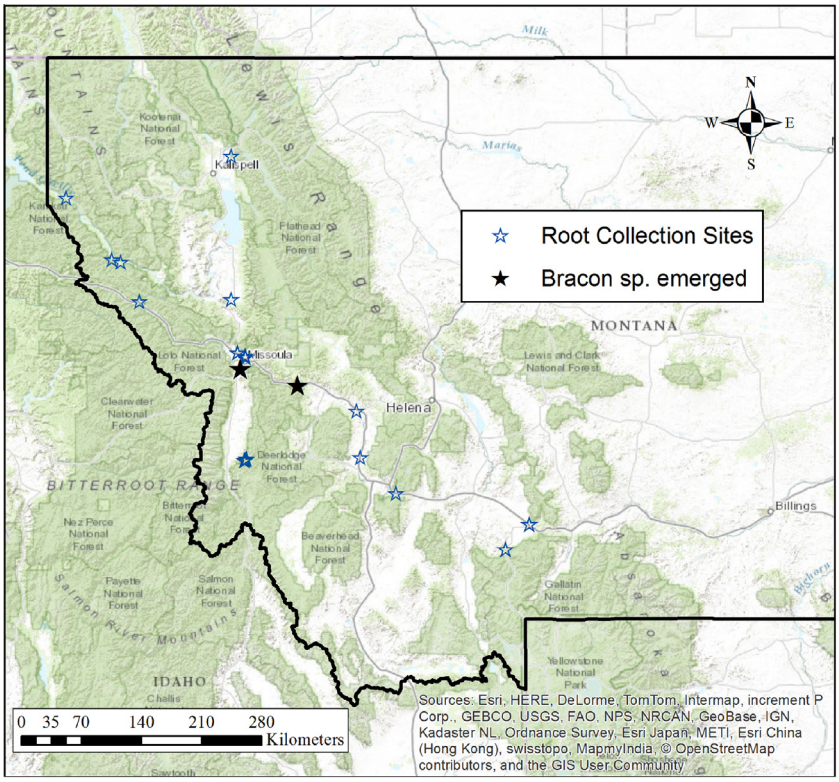


Fig. 3. *Centaurea stoebe* root collection sites from 2012. Solid stars are those sites where *Bracon* sp. emerged.

pupal or adult *P. carlinarum* (mean of 7.5 ± 0.84 SE per gall). Although *P. carlinarum* had the highest overall emergence compared to other parasitoids, its abundance was variable among sites. Assuming 7.5 parasitoids per *U. affinis* host, parasitism of the August collections ranged from 0% to 75% among locations (Table 2). In the September/October and May collections, there were a number of sites where individuals of *P. carlinarum* emerged, but no *U. affinis* (although galls were found during dissection) suggesting 100% parasitism of *U. affinis* larvae or high overwinter mortality of *U. affinis*. Mean percent parasitism rates in Table 2 were calculated assuming 100% parasitism where no parasitoids emerged.

3.3.2. S. brevicornis

Only a few individuals of *S. brevicornis* emerged from 2012 and April 2013 collections. The August and September 2013 seedhead collections had very low insect emergence in general, and only one *S. brevicornis* individual emerged. Examination of this seedhead revealed typical *Larinus* spp. damage. There was no distinguishable exoskeleton left by the original occupant, but since no evidence of other biocontrol agents was found, we assumed that *S. brevicornis* parasitized a *Larinus* spp. larva or pupa.

3.3.3. Bracon sp.

Bracon sp. emerged from roots of two of the 19 sites (Table S1, Fig. 3) in 2012 and again in 2013. During dissection of this root material, we observed two roots that contained several cocoons (one had four and the other seven) inside boring tunnels characteristic of *C. stoebe* root biocontrol agents. We identified these as puparia of the *Bracon* sp. In both cases, an *A. zoegana* head capsule was present with the braconid puparium. We therefore concluded that the *Bracon* sp. most likely attacked *A. zoegana*. Since multiple *Bracon* puparia were found in both boring tunnels, we concluded that this *Bracon* sp. was gregarious. Parasitism at the two sites were estimated at 20% and 29%.

4. Discussion

Ten of the thirteen biological control agents established against *C. stoebe* emerged from plant material collected in western Montana. Some of these are well established throughout the range of *C. stoebe* in Montana and the rest of North America (i.e., both *Urophora* species and *L. minutus*, Winston et al., 2010), but others are less common in Montana and were not thought to be established in the state (i.e., *T. virens* and *P. inspersa*, Julien and Griffiths, 1998; Winston et al., 2010). Although these agents were relatively rare in samples, they appeared to be widely distributed in western Montana.

At least four parasitoid species emerged that were not previously known to be associated with *C. stoebe* biological control agents in western Montana: *P. elevatus*, *P. carlinarum*, *S. brevicornis*, and *Bracon* sp. To date, only parasitoids attacking the two species of *Urophora* gall flies have been recorded (Gillespie, 1983; Kovach, 2004; Lang and Richard, 1998; Lang et al., 2003; Marshall, 2007; Marshall et al., 2004). This is the first study to find evidence of *A. zoegana* and *Larinus* spp. parasitism in their introduced range. Several studies have documented parasitism of *U. affinis* (Gillespie, 1983; Lang and Richard, 1998; Marshall, 2007), but this is the first record of *P. carlinarum* utilizing *U. affinis*.

Although not directly observed in this study, we strongly suspected that *P. cardui* was parasitizing *U. quadrifasciata*, given previous research in Tennessee and Michigan documenting this host–parasitoid relationship (Kovach, 2004; Marshall et al., 2004). The other species of *Pteromalus* identified, *P. elevates*, has been reared from *Centaurea* spp. in Europe and is known to attack many species of Tephritidae including species in the genera *Chaetorellia*, *Terellia*, and *Urophora* (Noyes, 2013). Therefore, we

suspect that this parasitoid was attacking these same genera of *C. stoebe* biocontrol agents in Montana. No affiliation with any *C. stoebe* biocontrol agent was found for the two unknown *Pteromalus* species, but species within this genus often attack similar hosts (Gibson et al., 1997), so it is possible they also parasitized the tephritids. The host association of *Mesopolobus* sp. is also unknown, but it could be the same *Mesopolobus* sp. found by Lang and Richard (1998) associated with *U. affinis* in Montana. Similarly, it is possible that *E. vesicularis* is the same species that Gillespie (1983) found attacking *U. affinis* in Montana and Idaho.

Of the nine parasitoid species emerging from the *C. stoebe* plant material, *S. brevicornis*, *E. vesicularis*, and *Bracon* sp. were uncommon but widespread across collection sites in western Montana. Since these parasitoids were not locally abundant, they likely have little impact on populations of *C. stoebe* biocontrol agents. Although *Bracon* sp. emerged in relatively high numbers compared to its host *A. zoegana* in 2013 (estimated 20% and 29% parasitism), these rates are comparable to those in its native range (10% to 45% larval mortality by a single parasitoid) (Müller et al., 1988). These observations suggest that *A. zoegana* is not suffering unusually high mortality in Montana compared to its native range, but further research is needed to assess whether the observed level of parasitism may limit *A. zoegana* effectiveness.

In contrast to the other parasitoids, *P. carlinarum* and the Pteromalinae species emerged from 85% and 68% of all seedhead collections, respectively. Although emergence varied greatly between sites and collection dates, estimated levels of parasitism by *P. carlinarum* were extremely high at some locations, reaching 100% parasitism of overwintering *U. affinis* larvae. Although such levels of parasitism could severely impact *U. affinis* population persistence at these sites, immigration from areas with low parasitism may compensate, since *U. affinis* is very mobile (Winston et al., 2010). In addition, factors such as desiccation or freezing, which may have been exacerbated under laboratory conditions, could have contributed to unusually high *U. affinis* larval mortality, and dissections infrequently revealed dead and shriveled *U. affinis* larvae within galls. Given these qualifications, the widespread presence of *P. carlinarum* in locations where it was previously not encountered, and the large number of cases of 100% parasitism in the spring suggest that *P. carlinarum* may represent a real and possibly growing threat to *U. affinis* populations.

The suite of parasitoids associated with the flower-feeding agents in this study was quite similar to those found in previous studies in Montana and Idaho (Turner et al., 1990; Lang and Richard, 1998; Gillespie, 1983). Although we were not able to verify host associations for several of the parasitoids we found, if we assume (as discussed above) that parasitoids we found had the same hosts as determined in previous studies, the inventory of parasitoid species attacking *U. affinis* in 2012–13 was nearly the same as 20–30 years ago, with the notable exception being the appearance of *P. carlinarum*. Since introduced organisms have been shown to accumulate natural enemies over time (Cornell and Hawkins, 1993; Flory and Clay, 2013), we expected to find an increase in the number of parasitoids utilizing *U. affinis*, but the lack of change may simply be due to an inadequate amount of time for parasitoids to evolve behavioral, phenological or ecological specializations to be able to utilize the new host. The change our collections did reveal compared to previous studies from this region (Turner et al., 1990; Lang and Richard, 1998; Gillespie, 1983) was the surprisingly large numbers of individuals of some parasitoid species, particularly *P. carlinarum* and Pteromalus spp. These previous studies attributed insignificant mortality to parasitism because so few parasitoid individuals were encountered in well-established *Urophora* populations. Although differences in methodology certainly may account for some of the discrepancies in the level of parasitism, our study suggests that parasitism has increased over the intervening period.

In conclusion, nine parasitoids were reared from *C. stoebe* plant material collected in western Montana and four of these are newly-reported associations with *C. stoebe* biological control agents. Host–parasitoid associations were verified for three of the nine parasitoid species: *U. affinis* – *P. carlinarum*, *Larinus* spp. – *S. brevicornis*, and *A. zoegana* – *Bracon* sp. High abundance and widespread distribution make *P. carlinarum* and Pteromalinae spp. parasitoids of special concern and deserving of future research attention. The construction of demographic life-tables, in particular, would enable a better understanding of the population dynamics of both biological control agents and parasitoids.

Contribution of authors

All authors contributed to study design. C.R.H.S and J.L.L. conducted field and lab work. C.R.H.S. performed data analysis and wrote the manuscript with assistance from all authors.

Acknowledgments

This study would have been unsuccessful without the parasitoid identification provided by: Dr. Mike Ivie, Montana State University; Dr. Jim Woolley, Texas A&M; Dr. Steve L. Heydon, R.M. Bohart Museum of Entomology, University of California-Davis; Dr. Roger Burks, University of California-Riverside; Dr. Robert Wharton, Texas A&M; Dr. Gary Gibson, Canadian National Collection of Insects, Arachnids and Nematodes; and Dr. David Wahl, American Entomological Institute. Thank you also to all the individuals who helped with field work, lab work, and statistics. This project was funded by a Grant from the United States Department of Agriculture-National Institute of Food and Agriculture (USDA-NIFA), award #2012-67014-19365.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biocontrol.2015.04.001.

References

Boughton, A.J., Kula, R.R., Gates, M., Zhang, Y., Nunez, M., O'connor, J., Whitfield, J.B., Center, T.D., 2012. Parasitoids attacking larvae of a recently introduced weed biological control agent, *Neomusotima conspurcatalis* (Lepidoptera: Crambidae): key to species, natural history, and integrative taxonomy. Ann. Entomol. Soc. Am. 105, 753–767.

Cornell, H.V., Hawkins, B.A., 1993. Accumulation of native parasitoid species on introduced herbivores: a comparison of hosts as natives and hosts as invaders. Am. Nat. 141, 847–865. http://dx.doi.org/10.1086/285512.

Flory, S.L., Clay, K., 2013. Pathogen accumulation and long-term dynamics of plant invasions. J. Ecol. 101, 607–613. http://dx.doi.org/10.1111/1365-2745.12078.

Gibson, G.A., Huber, J.T., Woolley, J.B. (Eds.), 1997. Annotated keys to the genera of Nearctic Chalcidoidae (Hymenoptera). National Research Council of Canada, Ottawa, Ontario, 794 pp.

Gillespie, R.L., 1983. Bionomics of Urophora affinis Frauenfeld, and U. quadrifasciata Meigen (Diptera: Tephritidae) in northern Idaho. Master's Thesis, University of Idaho.

Groppe, K., 1990. *Larinus minutus* Gyll. (Coleoptera: Curculionidae), a suitable candidate for the biological control of diffuse and spotted knapweed in North America, European Station Report. CAB International Institute of Biological Control.

Groppe, K., 1992. Larinus obtusus Gyll. (Col.: Curculionidae), a candidate for biological control of diffuse and spotted knapweed, European Station Report. CAB International Institute of Biological Control.

Herron-Sweet, C.R., 2014. Multi-trohpic interactions between the invasive plant *Centaurea stoebe*, insects and native plants. Master's Thesis, Montana State University.

Hill, M.P., Hulley, P.E., 1995. Host-range extension by native parasitoids to weed biocontrol agents introduced to South Africa. Biol. Control 5, 297–302.

Julien, M.H., Griffiths, M.W. (Eds.), 1998. Biological control of weeds: a world catalogue of agents and their target weeds, Fourth ed. CAB International Publishing, New York.

Kovach, A.L., 2004. Assessment of insects, primarily impacts of biological control organisms and their parasitoids, associated with spotted knapweed (*Centaurea stoebe* L. s. l.) in eastern Tennessee. Master's Thesis, University of Tennessee, Knoxville.

Lang, R.F., Richard, R.D., 1998. Native parasitoids attacking *Urophora affinis* Frauenfeld (Diptera; Tephritidae), an introduced biocontrol agent of spotted and diffuse knapweeds (*Centaurea* spp.) in the United States. Pan-Pac. Entomol. 74, 223–227.

Lang, R.F., Winkler, J., Hansen, R.W., 2003. Attack of *Urophora quadrifasciata* (Meig.) (Diptera: Tephritidae) a biological control agent for spotted knapweed (*Centaurea maculosa* Lamarck) and diffuse knapweed (*C. diffusa* Lamarck) (Asteraceae) by a prasitoid. *Pteromalus* sp. Gt. Lakes Entomol. 36, 16–24.

Littlefield, J.L., 1991. Parasitism of *Rhynocyllus conicus* Froelich (Coleoptera: Curculionidae) in Wyoming. Can. Entomol. 123, 929–932.

Marshall, J.M., 2007. Parasitism of *Urophora affinis* (Diptera: Tephritidae) by *Aprostocetus* sp. (Hymenoptera: Eulophidae) in Michigan. Gt. Lakes Entomol. 40, 166–168.

Marshall, J.M., Burks, R.A., Storer, A.J., 2004. First host record for *Pteromalus cardui* (Hymenoptera: Pteromalidae) on Urophora quadrifasciata (Diptera: Tephritidae) in spotted knapweed (*Centaurea biebersteinii*, Asteraceae) in Michigan, USA. Entomol. News 115, 273–278.

Mills, N., 2009. Parasitoids. In: Resh, V., Cardé, R. (Eds.), Encyclopedia of insects. Elsevier Science & Technology, Oxford, United Kingdom.

Müller, H., Schroeder, D., Gassmann, A., 1988. *Agapeta zoegana* (L.) (Lepidoptera: Cochylidae), a suitable prospect for biological control of spotted and diffuse knapweed, *Centaurea maculosa*, Monnet De La Marck and *Centaurea diffusa* Monnet De La Marck (Compositae) in North America. Can. Entomol. 120, 109–124.

Müller-Schärer, H., Schroeder, D., 1993. The biological control of *Centaurea* spp. in North America: do insects solve the problem? Pestic. Sci. 37, 343–353.

Noyes, J.S., 2013. Universal Chalcidoidae Database. Accessed 20 January 2014 at http://www.nhm.ac.uk/chalcidoids.

Paynter, Q., Fowler, S.V., Hugh Gourlay, A., Groenteman, R., Peterson, P.G., Smith, L., Winks, C.J., 2010. Predicting parasitoid accumulation on biological control agents of weeds. J. Appl. Ecol. 47, 575–582. http://dx.doi.org/10.1111/j.1365-2664.2010.01810.x.

Rice, P.M., 2014. INVADERS Database System (http://invader.dbs.umt.edu/). Div. Biol. Sci. Univ. Mont. Missoula, MT 59812-4824.

Stiling, P., 1993. Why do natural enemies fail in classical biological control programs? Am. Entomol. 39, 31–37.

Stinson, C.S.A., 1987. Investigations on *Cyphocleonus achates* (Fahr.) (Col.:Curculionidae), a possible biological agent of spotted knapweed (*Centaurea maculosa* Lam.) and diffuse knapweed (*C. diffusa* Lam.) (Compositae) in North America. CAB International Institute of Biological Control.

Story, J.M., Stougaard, R.N., 2006. Compatibility of two herbicides with *Cyphocleonus achates* (Coleoptera: Curculionidae) and *Agapeta zoegana* (Lepidoptera: Tortricidae), two root insects introduced for biological control of spotted knapweed. Environ. Entomol. 35, 373–378.

Swope, S.M., Satterthwaite, W.H., 2012. Variable effects of a generalist parasitoid on a biocontrol seed predator and its target weed. Ecol. Appl. 22, 20–34.

Turner, C.E., Grissell, E.E., Cuda, J.P., Casanave, K., 1990. *Microdontomerus anthonomi* (Crawford) (Hymenoptera: Torymidae), an indigenous parasitoid of the introduced biological control insects *Bangasternus orientalis* (Capiomont) (Coleoptera: Curculionidae) and *Urophora affinis* Frauenfeld (Dipt.). Pan-Pac. Entomol. 66, 162–166.

United States Department of Agriculture, 2013. Plants Database [WWW Document]. Nat. Resour. Conserv. Serv. U.S. Dep. Agric. URL http://plants.usda.gov.

Van Driesche, R.G., 1983. Meaning of "percent parasitism" in studies of insect parasitoids. Environ. Entomol. 12, 1611–1622.

Wehling, W.F., Piper, G.L., 1988. Efficacy diminution of the rush skeletonweed gall midge, *Cystiphora schmidti* (Diptera: Cecidomyiidae), by an indigenous parasitoid. Pan-Pac. Entomol. 64, 83–85.

Winston, R., Schwärzlander, M., Randall, C.B., Peardon, R., 2010. Biology and biological control of knapweeds. Forest Health Technology Enterprise Team. FHTET-2010-01.